In vivo and in vitro expression of canine distemper viral proteins in dogs and non-domestic carnivores

Brief Report

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Summary. The occurrence of the nucleo-, phospho-, matrix, fusion, and hemagglutinin proteins of the canine distemper virus (CDV) was investigated immunocytochemically in the brains of 3 dogs, 6 stone martens, 1 polecat, and 1 weasel. In addition, viral protein expression was studied in primary brain cell cultures of the 3 dogs after co-cultivation with Vero cells. Immunohistochemically, only minor differences, restricted to the H-4 epitope, were noted between the various species and CDV isolates. The data presented indicate that the mustelid virus is antigenically not distinct from the canine morbillivirus.

Canine distemper virus (CDV) belongs to the genus morbillivirus, a member of the family Paramyxoviridae [14]. CDV, pathogenic for animals of the orders carnivora, suborder fissipedia, and artiodactyla, family tayassuidae, causes systemic illness with or without central nervous system (CNS) affection [3, 15]. In dogs, CNS lesions can be categorized in acute encephalopathy, acute encephalitis, subacute to chronic demyelinating encephalitis (CDE), old dog encephalitis (ODE), and post-vaccinal canine distemper encephalomyelitis [13, 15]. CDV possesses 6 structural proteins comprising 3 core proteins, consisting of the nucleo-(N), phospho-(P), and large (L) protein, and 3 envelope polypeptides represented by the matrix (M), fusion (F), and hemagglutinin (H) protein [10, 14, 17]. Antigenic variations among CDV strains occur most frequently in the H protein [7, 18].

Following the mass mortality of harbour seals in the Northern and Baltic...
Seas during 1988, phocine distemper virus (PDV), a CDV-like virus, was isolated from diseased seals and has been classified as a separate member of the genus morbillivirus based on its antigenic and genomic properties [7, 9, 16]. The host spectrum of PDV comprises animals to the orders carnivora, suborder pinnipedia, and cetacea. Recently, a porpoise virus isolate was preliminary classified as “delphinoid virus” (DDV) using a panel of monoclonal antibodies (mAbs) directed against CDV, PDV, and DDV. The porpoise isolate exhibited several unique epitopes and other epitopes present on CDV and PDV were absent on the porpoise virus [21]. Repeated endemic outbreaks of distemper in the dog population have raised the question of the emergence of new field strains of CDV [1, 11]. It is well known that martens, foxes, and badgers are susceptible to CDV and they may serve as a virus reservoir [6, 20]. Furthermore, previous studies showed that mustelids are susceptible to CDV and PDV [12, 18].

The aim of this study was to investigate the expression of 5 CDV-specific proteins and their epitopes a) in the CNS of dogs and mustelids and b) in cocultures of canine primary brain cells with Vero cells.

Three dogs with naturally occurring canine distemper were used in the study. Dog 1, a 2 month-old female mixed-breed dog, presented neurologic dysfunctions only. Dog 2, a 6 month-old female mixed-breed dog, showed purulent rhinitis and conjunctivitis, hyperkeratosis of the foot pads and snout and ataxia. The third dog, a 4 month-old male shepherd dog, originating from Turkey, exhibited signs of respiratory, gastrointestinal and neurologic disease. Nothing was known about the vaccination records of dog 1 and 3. Dog 2 had received a pentavalent vaccine (incl. distemper) once. Despite intensive supportive treatment, the condition of the animals deteriorated and they were killed. Eight CDV-positive mustelids, 6 stone martens, 1 polecot, and 1 weasel were kindly provided by Dr. M. Adami (Staatliches Institut für Gesundheit und Umwelt, Abt. Veterinärmedizin, Saarbrücken, Federal Republic of Germany). Clinical histories were only sketchy; some animals showed loss of natural shyness, others were killed by dogs or found dead near human settlements. Immediately after death, the brains of the dogs were removed aseptically and one hemisphere was processed for virus isolation. Due to advanced autolysis of the mustelid carcasses, virus isolation was not attempted in these animals. The remaining hemisphere of the canine CNS and the brains of the mustelids were fixed in 10% non-buffered formalin and embedded in paraffin, in addition, tissue samples were embedded in O.C.T. and quick frozen in liquid nitrogen as described [2].

For virus isolation, single cell suspensions and explant cultures were established from cerebrum, cerebellum, and medulla oblongata. Cells were cultured in 25 and 75 cm² flasks and maintained in Eagle’s MEM with Earle’s salts, supplemented with 10% fetal calf serum and antibiotics as described [4]. The primary brain cell cultures were co-cultivated with African green monkey kidney (Vero) cells 12 (dog 1), 13 (dog 3), and 18 (dog 2) days after seeding. Following 5 (dog 1), 6 (dog 2), and 4 (dog 3) passages, cells were grown on 4-chamber slides and confluent monolayers were fixed in acetone as described [2].